

In The Specification

Please amend page 1, lines 1 through 2 as follows:

**METHOD AND COMPOSITION COMPRISING AUTOLOGOUS ID PROTEIN  
FOR ALTERING A B CELL MEDIATED MALIGNANCY PATHOLOGY**

Please delete the paragraph at page 35, lines 18 and 19, as follows:

~~Further definitions and characterizations of low-grade lymphomas can be found on the Internet at [http://rituxan.com/professional/clinical\\_information/class/index.html](http://rituxan.com/professional/clinical_information/class/index.html).~~

Please amend the paragraph at page 49, lines 19 through 21, as follows:

b. Total RNA Preparation: Total RNA from homogenized lymph node cells was isolated using RNeasy Kit RNeasy Kit® (Qiagen) as per manufacturer's instruction. Total RNA was quantitated by spectrophotometry.

Please amend the paragraph at page 49, lines 22 through 25, as follows:

c. cDNA Synthesis: Approximately 2.0 µg total RNA was used as template for first strand cDNA synthesis using the ~~SuperScript~~ SuperScript™ Preamplification System (~~GIBCO~~ GIBCO™-BRL) according to manufacturer's recommendation. Oligo(dT) provided with the kit was used to prime the cDNA.

Please amend the paragraph at page 50, lines 14 through 19, as follows:

One microliter of the cDNA reaction (representing 5% of the total cDNA reaction volume) was amplified for 35 cycles in 50 µl volume using the ~~HotStarTaq~~ HotStarTaq™ Master Mix Kit (Qiagen). Cycling conditions: 95 °C 15 min, 65 °C 4 min, 72 °C 1 min, followed by 94 °C 1 min, 61 °C 30 sec, 72 °C 1 min for 34 cycles; and a final extension step at 72 °C for 7 min. A 10 µl aliquot of each reaction is analyzed by electrophoresis on a 1% agarose gel with ethidium bromide.

Please amend the paragraph at page 53, lines 3 through 19, as follows:

e. Cloning and Sequencing of PCR Products: PCR products from reactions determined to contain the tumor specific variable sequences for heavy and light chains were cloned directly into plasmid pCR2.1-TOPO as per manufacturer's recommendations, and introduced into ~~Top10~~ Top10<sup>TM</sup> competent *E. coli* cells (Invitrogen). Twenty four miniprep DNA plasmids were prepared from carbenicillin resistant bacterial colonies using the ~~QIAprep~~ QIAprep® Spin Miniprep Miniprep<sup>TM</sup> Kit (Qiagen), and quantitated by spectrophotometry. Two hundred ng of each plasmid was sequenced using the ~~Cy5/Cy5.5 Dye Primer Cycle Sequencing Kit~~ Dye Primer Cycle Sequencing Kit<sup>TM</sup> (Visible Genetics). Following the completion of the sequencing reactions, samples were electrophoresed on the ~~OpenGene~~ OpenGene<sup>TM</sup> Automated DNA Sequencing System and the data was processed with ~~GeneObjects~~ GeneObjects<sup>TM</sup> software package (Visible Genetics). Additional analysis including sequence alignments were performed using the ~~SEQUENCHER~~ SEQUENCHER<sup>TM</sup> Version 4.1.2 DNA analysis software (GENE Codes Corp.). A V-region derived sequence could be considered tumor specific if it was present in 75% of the samples, for example, if 18 or greater of the 24 form a consensus group when analyzed using the above software utilizing the default parameters. Two independent biopsy samples would be compared when available.

Please amend the paragraph at page 54, lines 1 through 25, as follows:

f. cDNA Synthesis and Generation of 5' RACE Products: Due to the occurrence of mutations in the V<sub>H</sub> and V<sub>L</sub> sequences, it is not possible at times to identify tumor-specific immunoglobulin rearrangements. As an alternative to the sequence-specific PCR strategy *supra*, one can employ a 5' RACE PCR strategy to identify tumor specific immunoglobulin (Ig) rearrangements. All steps for first strand cDNA synthesis to the generation of Ig specific PCR products are performed according to manufacturer's directions (5' RACE system for Rapid Amplification of cDNA Ends, version 2.0, ~~Gibco~~ GIBCO<sup>TM</sup> BRL), with slight modification. Approximately 2.5 µg of total RNA is used as template for each first strand cDNA synthesis in the presence of specific antisense primers complimentary to the immunoglobulin heavy and the light chains' constant region utilized by the B cell population of interest (SEQ ID NO:69 for IgG, SEQ ID NO:70 for IgM, SEQ ID NO:71 for C<sub>κ</sub>, and SEQ ID NO:72 for C<sub>λ</sub>). The cDNA reactions are purified over ~~GlassMAX~~ GlassMAX<sup>TM</sup> spin cartridges, generating a final volume

of 50 µl each. A 10 µl aliquot of each purified cDNA is oligo(dC) tailed with terminal deoxynucleotidyl transferase in a 25 µl volume, generating the templates to be used for subsequent PCR reactions. The PCR set up utilizes an upstream primer containing a poly(G) track provided by the manufacturer and an Ig specific antisense primer interior to that used for cDNA first strand synthesis (SEQ ID NO:73 for IgG, SEQ ID NO:74 for IgM, SEQ ID NO:75 for C<sub>κ</sub>, and SEQ ID NO:76 for C<sub>λ</sub>). Five µl of template is amplified in a 50 µl volume as follows: 95 °C for 15 min, 55 °C for 4 min, 72 °C for 1 min, followed by 94 °C for 1 min, 55 °C for 30 sec, 72 °C for 1 min for 34 cycles, and a final extension step at 72 °C for 7 min. The final PCR products are separated by electrophoresis on a 1% agarose gel with ethidium bromide and the band of the appropriate size (~500-600 bp) is isolated ~~are~~ and cloned into the pCR2.1-TOPO plasmid as described in 1e, *supra*.

Please amend the paragraph at page 55, lines 3 through 26, as follows:

a. Cloning of Secretory Signal Sequences into p2Bac: The base vector for the pTRABacHuLC<sub>κ</sub>HC<sub>γ1</sub> and pTRABacHuLC<sub>λ</sub>HC<sub>γ1</sub> constructs was p2Bac (Fig. 2, SEQ ID NO:5, Invitrogen, Carlsbad, CA). Two secretory signal sequences were cloned into this base vector, and the first intermediate baculovirus expression vector p2BacM was created. In general, the vector p2Bac was first modified utilizing complimentary oligonucleotides encoding the amino terminal domain of the honey bee melittin secretory signal sequence positioned to be under transcriptional control of the baculoviral AcNPV P10 promoter. For melittin sequence cloning, 2 µg p2Bac was digested with Not I and Spe I for 4 hours at 37 °C. The linear vector was purified following electrophoresis through a 1% agarose gel using ~~Qiaex~~ Qiaex® II resin (Qiagen, Chatsworth, CA). The purified DNA was then eluted with 50 µl water and the DNA concentration was determined. One µg each of primers Me1S/N (SEQ ID NO:15) and MeIN/S (SEQ ID NO:16) were mixed in 10 µl digestion buffer M (Roche Molecular Biochemicals, Indianapolis, IN), and heated to 70 °C for 5 min, then cooled to room temperature to anneal complimentary primers. Ten percent of the annealed primers was digested in 20 µl reaction with Not I and Spe I for 4 hours at 37 °C, and the digested primers were purified following electrophoresis through a 15% polyacrylamide gel with ~~Qiaex~~ Qiaex® H resin, and the concentration of the DNA for annealed primers was determined. The DNAs of p2Bac vector and annealed melittin fragment were

ligated at 1:10 vector to insert ratio. The ligation product was transformed using competent XL1-Blue *E. coli* (Stratagene, San Diego, CA) and plated on a LB-carbenicillin agar plate for overnight growing at 37 °C. Miniprep colonies were prepared by standard protocols, and the plasmids were sequenced to check the construction. The resulting vector p2BacM contained the melittin secretory signal sequence.

Please amend the paragraph at page 56, lines 1 through 22, as follows:

The p2BacM vector was further modified similarly to encode for the amino terminal domain of the human placental alkaline phosphatase secretory signal sequence under transcriptional control of the AcNPV polyhedron promoter, creating a second intermediate baculovirus expression vector p2BacMA. The procedure used to introduce the alkaline phosphatase sequence was generally cloned as follows: 2 µg p2BacM plasmid was digested with Bam HI and Eco RI, the linear vector was gel purified from agarose gel with ~~Qiaex~~ Qiaex® II resin and eluted in 50 µl water. The DNA concentration of the vector was determined. One µg each of primers APB/E (SEQ ID NO:17) and APE/B (SEQ ID NO:18) were mixed in 10 µl digestion buffer M, and heated to 70 °C for 5 min and then cooled down to room temperature to anneal complimentary primers. Ten percent of the annealed primers was digested in a 20 µl reaction with Bam HI and Eco RI for 4 hours at 37 °C. The digested primers were then purified from 15% polyacrylamide gel with ~~Qiaex~~ Qiaex® II resin. The DNA concentration of the digested primers was also determined. The linear p2BacM vector and alkaline phosphatase fragment were then ligated at 1:10 vector to insert ratio, and the ligation product was transformed using competent XL1-Blue *E. coli* and plated on a LB-carbenicillin agar plate for overnight growing at 37 °C. Miniprep colonies were prepared and the plasmids were sequenced to check the construction. The resulting intermediate vector p2BacMA would contain a secretory signal sequence for a human placental alkaline phosphatase. The p2BacMA plasmid was further transformed into SCS-110 *E. coli* strain (Stratagene) lacking *dcm* methylase activity for subsequent cloning of the κ constant region into methyl-sensitive Stu I site.

Please amend the paragraph at page 57, lines 8 through 21, as follows:

c. Amplification of 9F12  $\kappa$  and IgG <sub>$\gamma$ 1</sub> constant region fragments: Total RNA from 9F12 cells (ATCC#HB8177) was extracted using the ~~RNeasy-Kit~~ RNeasy Kit® (Qiagen) as per the manufacturer's instruction. A single stranded cDNA was synthesized using ~~SuperScript~~ SuperScript™ reverse transcriptase (~~GIBCO~~ GIBCO™ BRL, Rockville, MD) with oligo(dT) primers. One twentieth of the synthesized single strand cDNA was amplified in 100  $\mu$ l PCR reactions with [Expand High Fidelity] Expand High Fidelity™ Taq (Roche) using  $\kappa$  and IgG <sub>$\gamma$ 1</sub> specific oligonucleotides (SEQ ID NO:21 plus SEQ ID NO:22 and SEQ ID NO:19 plus SEQ ID NO:20, respectively). The fragments from amplified 9F12 immunoglobulin were purified from 1.5% ~~SeaKem~~ SeaKem® agarose with ~~Qiaex~~ Qiaex® II resin and eluted with 50  $\mu$ l water. The DNA concentrations for the fragments were determined. The purified 9F12 immunoglobulin fragments were ligated separately into the TA-II (Invitrogen) PCR cloning vector. The ligation products were transformed using competent XLI-Blue *E. coli* and plated on a LB-carbenicillin agar plate for overnight growing at 37 °C. Miniprep colonies were prepared and the plasmid DNA was sequenced.

Please amend the paragraph at page 59, lines 6 through 9, as follows:

f. Addition of the  $\lambda$  Constant Region to the Vectors: Total RNA from purified peripheral blood lymphocytes (PBL) obtained from a chronic lymphocytic leukemia (CLL) patient displaying a  $\lambda$  light chain idiotype was extracted using the ~~RNeasy-kit~~ RNeasy kit® (Qiagen).

Please amend the paragraph at page 59, line 6, through page 60 line 6, as follows:

Approximately 2.0  $\mu$ g total RNA was used as template for first strand cDNA synthesis using the ~~SuperScript~~ SuperScript™ Preamplification System (Gibco BRL) according to manufacturer's recommendation. Oligo(dT) was used for priming. One twentieth of the synthesized single stranded cDNA was amplified in a PCR reaction using an upstream primer identical to a portion of the V $\lambda$  signal sequence (SEQ ID NO:54) and a downstream primer (SEQ ID NO:77) complimentary to the last several codons of the  $\lambda$  constant region as well as a portion of the 3' untranslated region. The PCR products were cloned into the pCRII vector (Invitrogen)

and sequenced to confirm identity. A plasmid containing the correct  $\lambda$  constant region sequence was chosen as a template for a second PCR. In this reaction a sense oligonucleotide, C $\lambda$ -5' (SEQ ID NO:78), containing an engineered Dra III restriction site, corresponding the sequence in the  $\lambda$  constant region immediately downstream of J $\lambda$  and a Hind III containing antisense oligonucleotide primer, C $\lambda$ -3' (SEQ ID NO:79) spanning the STOP codon immediately following the  $\lambda$  constant region were utilized. The resulting PCR product was cloned into the pCR2.1-TOPO vector and sequenced. A fragment containing the  $\lambda$  constant region sequence was released upon Hind III restriction from some of the plasmids, depending on orientation of the insert. This restriction fragment was gel isolated and cloned into pTRABacHuLC $\kappa$ HC $\gamma$ 1 (Figure 5A), following linearization following Hind III digestion, generating an intermediate plasmid containing both the  $\lambda$  and  $\kappa$  constant regions. Restriction of this plasmid with Stu I and Dra III resulted in the removal of the  $\kappa$  sequences. This linearized plasmid was then ligated with annealed complimentary primers  $\lambda$ -stuff 1 (SEQ ID NO:80) and  $\lambda$ -stuff 1' to generate the final version of pTRABacHuLC $\lambda$ HC $\gamma$ 1 (Figure 5B).

Please amend the paragraph at page 60, lines 10 through 21, as follows:

Using either pTRABacHuLC $\kappa$ HC $\gamma$ 1 or pTRABacHuLC $\lambda$ HC $\gamma$ 1, it was possible to insert genes for any V<sub>L</sub> region containing the unique cloning sequences Stu I and Dra III between the alkaline phosphatase signal sequence and the  $\kappa$  or  $\lambda$  constant region, and genes for any V<sub>H</sub> region containing the unique cloning sequences Spe I and Apa I between the melittin secretory signal sequence and the IgG $\gamma$ 1 constant region (See Figure 5A and 5B). The resulting expression vector could then be utilized for transduction into *Spodoptera frugiperda* (Sf 9) insect cells to produce recombinant budded baculovirus. The recombinant baculovirus was then serially amplified in Sf 9 cells to produce a high titer recombinant baculovirus stock. This high titer recombinant baculovirus stock was then used to infect *Trichoplusia ni* (~~High 5~~) High Five<sup>TM</sup> cells for subsequent chimeric IgG protein production. A list of all oligonucleotide primers used in the construction of pTRABacHuLC $\kappa$ HC $\gamma$ 1 or pTRABacHuLC $\lambda$ HC $\gamma$ 1 can be found in Table 2.

Please amend the paragraph at page 61, line 18, through page 62, line 2, as follows:

a. Light Chain Variable Region Insertion into Expression Vector: A PCR derived  $V_L$  product and 2  $\mu$ g of the corresponding pTRABacHuLC $\kappa$ HC $\gamma_1$  or pTRABacHuLC $\lambda$ HC $\gamma_1$  cassette vector digested with *Stu* I and *Dra* III. The 350 bp DNA fragment from the patient derived  $V_L$  region and the 8.4 kb fragment for the linear pTRABacHuLC $\kappa$ HC $\gamma_1$  or pTRABacHuLC $\lambda$ HC $\gamma_1$  vector were purified from agarose gel plugs with ~~Qiaex~~ Qiaex® II resin and eluted in 50  $\mu$ l water. The DNA concentrations for both fragments were determined and then the fragments ligated using Rapid Ligation kit (Roche). The ligation products were used to transform competent XL1-Blue *E. coli* which were subsequently plated on a LB-carbenicillin agar plate for overnight growing at 37 °C. Miniprep colonies were prepared and the recombinant DNA plasmids were verified by restriction analysis and sequencing. The resulting vector designated pTRABac(NHL- $V_L$ )LC $\kappa$ HC $\gamma_1$  or pTRABac(NHL- $V_L$ )LC $\lambda$ HC $\gamma_1$ .

Please amend the paragraph at page 62, lines 3 through 16, as follows:

b. Heavy Chain Variable Region Insertion into Expression Vector: A PCR derived  $V_H$  product and 2  $\mu$ g of the pTRABac(NHL- $V_L$ )LC $\kappa$ HC $\gamma_1$  or pTRABac(NHL- $V_L$ )LC $\lambda$ HC $\gamma_1$  cassette vector were digested with *Spe* I and *Apa* I. The 350 bp DNA fragment from the patient derived  $V_H$  region and the 8.8 kb fragment for the linear pTRABac(NHL- $V_L$ )LC $\kappa$ HC $\gamma_1$  or pTRABac(NHL- $V_L$ )LC $\lambda$ HC $\gamma_1$  vector were purified from agarose gel plugs with ~~Qiaex~~ Qiaex® II resin and eluted in 50  $\mu$ l water. The DNA concentrations for both fragments were determined and then the fragments ligated using Rapid Ligation kit (Roche). The ligation products were used to transform competent XL1-Blue *E. coli* which were subsequently plated on a LB-carbenicillin agar plate for overnight growing at 37 °C. Miniprep colonies were prepared and the recombinant DNA plasmids were verified by restriction analysis and sequencing. The resulting vector is designated pTRABac(NHL- $V_L$ )LC $\kappa$ (NHL- $V_H$ )HC $\gamma_1$ , pTRABac(NHL- $V_L$ )LC $\lambda$ (NHL- $V_H$ )HC $\gamma_1$  and is assigned a reference number corresponding to a patient, e.g., FV8786-001.

Please amend the paragraph at page 63, line 15, through page 64, line 16, as follows:

b. **Sf9 Cell Transfection and Recombination Assay:** The modified baculovirus expression vectors containing genes for  $V_H$  and/or  $V_L$  regions and genes encoding

immunoglobulin heavy and/or light chain constant regions were co-transfected into Sf9 cells using the ~~BacVector-3000~~ BacVector-3000™ transfection kit (Invitrogen). Ten individual plaques are picked from agarose overlays. Virus from isolated plaques are used to infect T-25 flasks seeded with Sf-9 cells at 50% confluency in 5 ml ESF-921 media. Clonal viral isolates amplified in T-25 flasks are tested by PCR, using two primers (SEQ ID NO:36 – TTTACTGTTT TCGTAACAGT TTTG) and (SEQ ID NO:37 – GGTCGTTAAC AATGGGGAAG CTG) to assure clonality of the isolated plaques and that there was no wild type virus contamination. In general, 200 ng recombinant transfer vector plasmid was co-transfected with triple-cut Bac-Vector-3000 as described in the ~~Bac Vector~~ Bac Vector® manual (Novagen) using the Eufectin lipid reagent supplied. This transfection mixture was subjected to serially 5-fold dilutions. One hundred microliter aliquots were plated in 60 mm tissue culture dishes containing  $2.5 \times 10^6$  adherent Sf9 cells. After 1 hour, cells were overlaid with 4 ml of a 1% agarose solution in ESF-921 culture medium. Ten individual clones were picked from the transfected cells grown in agarose overlays after staining for live cells using Neutral Red (Sigma, St. Louis, MO) at t=144 hours post transfection. Virus was eluted from plaque plugs overnight in 1 ml ESF-921 media. T-25 flasks were seeded with Sf-9 cells at 50% confluency in 5 ml ESF-921 media, and infected with 0.5 ml of eluted clonal virus. Ninety-six hours post infection, 0.5 ml media was removed from T-25 flasks; the cells were removed by centrifugation and the supernatant was assayed for immunoglobulin activity by dot blotting on nitrocellulose. The absence of wild type virus was also tested by PCR as follows.

Please amend the paragraph at page 64, line 17, through page 65, line 5, as follows:

Infectious supernatant (10 µl) containing recombinant baculovirus was added to 90 µl of lysis buffer containing 10 mM Tris pH 8.3, 50 mM KCl, 0.1 mg/ml gelatin, 0.45% ~~Nonidet~~ Nonidet® P-40, and 0.45% Tween-20, containing 6 µg Proteinase-K. The mixture was heated for 1 hour at 60 °C and the Proteinase-K was denatured by incubation at 95 °C for 10 min. Twenty five µl of the heated mixture was removed to a fresh PCR tube after cooling, and another 25 µl of the mixture containing 10 mM Tris pH 8.3, 50 mM KCl, 0.1 mg/ml gelatin, 0.45% NP-40, 0.45% Tween-20, 400 µM each dNTP, 5 mM MgCl<sub>2</sub>, 50 pM each PCR primer (final), and 2.5 U Taq polymerase (Roche) was added. The viral DNA was amplified for 40 cycles at: 92 °C for 1 min., followed by 58 °C for 1 min. and 72 °C for 1 min. The recombinant baculovirus

primers PH forward (SEQ ID NO:36) and PH reverse (SEQ ID NO:37) were used to amplify the polyhedron locus expressing the light chain gene. PCR products were analyzed following electrophoresis through an agarose gel. Recombinant baculovirus would amplify a 1300 bp fragment, while wild type baculovirus would produce a ~ 800 bp fragment with these primer sets. Recombinant virus contaminated with wild type virus would amplify both fragment sizes.

Please amend the paragraph at page 66, lines 17 through 16, as follows:

Cells and debris were removed by centrifugation for 60 min. at approximately 5,000 x g, followed by filtration through a 0.2µ PES sterile filter unit. Chimeric proteins were purified from cleared tissue culture media by affinity chromatography with a Protein-A HiTrap™ ~~High-Trap~~ cartridge (Amersham Pharmacia, Piscataway, NJ), followed by ion-exchange chromatography utilizing FPLC technology (Amersham Pharmacia). The purified chimeric proteins were size separated and buffer exchanged into PBS by FPLC chromatography. All reagents used for protein purification were of USP biotechnology grade (GenAr, Mallinckrot Baker, Parris, KY) and endotoxin tested by the manufacturer. Sterile USP grade water was used to make all buffers and other solutions. Buffers and other solutions were prepared in a biological safety cabinet, and filter sterilized through 0.2 µm PES filter units.

Please amend the paragraph at page 66, line 17, through page 67, line 3, as follows:

**a. ~~Protein-A Sepharose~~ Protein A Sepharose™ Affinity Purification of the Chimeric Proteins:** Tissue culture medium was removed from growing culture flasks and spun for 60 min. at 5,000 x g to sediment cells and debris. The supernatant was sterilized by filtration using a 0.2µ PES filter unit. Tris buffer (1M, pH 7.4) was added to the filtered medium containing V<sub>H</sub> and/or V<sub>L</sub>-immunoglobulin chimeric proteins to a final concentration of 20 mM. The buffered tissue culture supernatant was loaded onto a 5 ml HighTrap recombinant ~~Protein-A Sepharose~~ Protein A Sepharose™ affinity cartridge at a flow rate of 1 to 5 ml/min with a P1 peristaltic pump (Amersham Pharmacia) collecting the flow-through in a clean flask. The column was washed with 25 ml PBS (pH 7.4) at 5 ml/min. The direction of the flow was reversed and the column was washed with an additional 25 ml PBS. The column was eluted in reverse at 1 ml/min with 0.05 M citric acid (pH 3.5) collecting 1 ml fractions. Other protein

columns including but not limited to protein G, protein L, or any proteins that are able to bind to an immunoglobulin binding domain could be used in the same manner.

Please amend the paragraph at page 67, lines 4 through 13, as follows:

**b. Ion Exchange Chromatography:** A 5 ml ~~High Trap~~ HiTrap<sup>TM</sup> SP Sepharose cation exchange cartridge was equilibrated with 50 ml of 25 mM citric acid (pH 3.5) and 20 mM NaCl. The Protein A eluted V<sub>H</sub> and/or V<sub>L</sub>-IgG chimeric proteins were loaded directly onto the equilibrated High Trap SP Sepharose column using a peristaltic pump at a flow rate of 1 ml/min. The column was washed with 25 ml 50 mM citric acid (pH 3.5) and 20 mM NaCl (Buffer A) at 2 ml/min. The column was eluted with a linear gradient (0% Buffer B to 100% Buffer B) to collect 1 ml fractions at 1 ml/min. (Buffer B = 100 mM Na carbonate (pH 10.0) and 1M NaCl). The ion exchange eluted fractions containing V<sub>H</sub> and/or V<sub>L</sub>-IgG chimeric proteins were analyzed spectrophotometrically by their OD280. The peak fractions were pooled.

Please amend the paragraph at page 67, lines 14 through 18, as follows:

**c. Size Exclusion Chromatography:** The pooled Ig fraction from SP ion-exchange was then loaded onto a ~~Hi-Prep~~ HiPrep<sup>TM</sup> Sephacryl 26/60 S200 Hi Resolution column (Pharmacia) that had been equilibrated in 5 column volumes of PBS (pH7.2) following a pre-wash in 100 ml sterile water. The chimeric Ig proteins were eluted in PBS at a flow rate of 0.5 ml/min and collected in 1 ml fractions. The major Ig peak was apooleed a sterile filtered through a 0.2 $\mu$  filter.

Please amend the paragraph at page 67, line 22, through page 68, line 9, as follows:

Once purified, the idiotypic protein was conjugated to GMP grade KLH (~~VACMUNE~~ VACMUNE®, Biosyn Corporation) via glutaraldehyde crosslinking. At least 5 mg of purified, sterile idiotypic protein as described, *supra*, was combined with an equal weight of KLH in a sterile 15 ml conical tube and the final volume was adjusted to 9 ml in PBS. One ml of 1% glutaraldehyde (25% Grade 1 aqueous solution, Sigma) was added dropwise to a final concentration of 0.1%. The tube was then slowly rocked for 4 hours at room temperature. The

conjugate was dialyzed in sterile ~~DispoDialyzers~~ DispoDialyzers® (Spectrum Labs) against 2 liters sterile PBS, with three buffer changes over at least 24 hours in a biological safety hood. The final IgG/KLH conjugate in PBS is aseptically removed from the dialysis chambers and transferred into a sterile tube, mixed, then aliquoted in vials. Each vial of final product was labeled with the lot number, patient identifier, vial number and date vialled. Ten percent of the final vialled lot was tested for sterility and a vial was tested for the presence of endotoxin. One vial was retained for archival purposes.

Please amend the paragraph at page 68, line 11, through page 69, line 8, as follows:

**a. DNA Sequence of Baculovirus Containing Production Lot Supernatant:** A 1 ml aliquot of sample of infected insect cell production culture supernatant was harvested and cleared of cellular debris by spinning at 3000 rpm for 5 min in a desktop centrifuge. At least 0.1 ml of this cleared supernatant containing baculovirus particles was combined at a volume ratio of 1 to 9 with lysis buffer (10mM Tris pH 8.3, 50 mM KCl, 0.1 mg/ml gelatin, 0.45% ~~Nonidet~~ Nonidet® P-40, and 0.45% Tween-20), subjected to proteolysis with proteinase K (final concentration 60 µg/ml) for 1 h at 60 °C, followed by denaturation for 15 min at 95 °C. Twenty-five µl of this lysate was then combined with an additional 25 µl of the above lysis buffer containing 400 µM each dNTP, 5 mM MgCl<sub>2</sub>, 25 pmol forward and reverse oligonucleotide primers (see Table 3; SEQ ID NO:34 and SEQ ID NO:31 for V<sub>H</sub> Identification and SEQ ID NO:35 and SEQ ID NO:36 for V<sub>L</sub> identification, respectively), and 2.5 U Taq polymerase (Roche). Cycling conditions for the PCR of V<sub>L</sub> are: initial denaturation for 2 min at 92 °C, followed by 40 cycles of 1 min each at 92 °C, 58 °C, and 72 °C, with a final extension of 7 min at 72 °C. For the PCR of V<sub>H</sub>, cycling conditions are the same except that the annealing temperature is 64 °C. PCR products were assessed for expected size and quantity by agarose gel electrophoresis. Subsequently, two or more nested primers were used to directly sequence the PCR products. (See Table 3; SEQ ID NO:30 and SEQ ID NO:34 for V<sub>H</sub> identification, SEQ ID NO:28 and SEQ ID NO:35 for V<sub>K</sub> identification, and SEQ ID NO:88 and SEQ ID NO:35 for V<sub>L</sub> identification, respectively.) The complete V<sub>H</sub> and V<sub>L</sub> nucleotide sequences was determined using the the ~~OpenGene~~ OpenGene™ Automated DNA Sequencing System (Visible Genetics) and sequencing analysis software, as described above and compared with the V-gene sequences of the pTRABac(NHL-FV-8786-XXX) vector corresponding to that patient's idiotype.

Please amend the paragraph at page 69, lines 11 through 17, as follows:

b. **Superose Superose™ 6 Gel Filtration Chromatography:** Gel filtration chromatography of the purified Id was performed to assess protein purity. Gel filtration chromatography was performed using a ~~Superose~~ Superose™ 6 HR 10/30 FPLC column (Amersham Pharmacia) with PBS as the liquid phase. Peak integration was performed on the largest 20 peaks by the FPLC software using the following criteria to reject a peak from being included in area evaluation: height less than 0.01 Au; width less than 0.05 ml; area less than 0.01 Au/ml. Fractions of each column run were collected and assayed for human immunoglobulin specific activity by capture ELISA, and compared to the OD<sub>280</sub> chromatogram.